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OBSERVATIONS ON THE FEEDING OF FLEAS UNDER CONDITIONS OF FORCED FEEDING THROUGH CAPILLARY TUBES CONTAINING THE PLAGUE MICROBE

[Following is the translation of an article by A. N. Alekseyev, V. A. Bibikova, and N. N. Khrustselevskaya, Central Scientific-Research Disinfection Institute of the USSR Ministry of Public Health, Moscow, and the Central Asian Scientific-Research Antiplague Institute, Alma-Ata, published in the Russian-language periodical Parazitologiya (Parasitology) II, 2, 1968, pp 115—123.]

Investigations were made of the rate of feeding and volume of engorged liquid containing a proportioned amount of plague microbes by Xenopsylla gerbilli minax Jord. and Ceratophyllus laeviceps Wagn. fleas under conditions of forced feeding. The fate of the causative agent was followed under various conditions of feeding.

Development of a method for the individual proportioned feeding of fleas with plague microbes (Alekseyev, 1965, Alekseyev, 1967) made it possible to conduct a number of preliminary observations of the rhythm and rate of feeding of Xenopsylla gerbilli minax Jord., Ceratophyllus laeviceps Wagn., and X. cheopis Rothsch. fleas, to determine with an accuracy to 0.005 ml the volume of liquid engorged by an individual specimen, and taste preference of fleas, and to follow the fate of the microbes in fleas and the survival of specimens which had been infected with various doses of plague culture.

As criteria for the suitability of nutritive liquid for the feeding of fleas it was accepted to use the rate of saturation and volume of engorged liquid. Data concerning the rate of feeding (absorption of 0.045 ml) were treated statistically. In the process of treating the material it turned out that the only statistically reliable data were obtained under conditions when the fleas were divided into 2 groups based on rate of feeding - up to 60 seconds and more than 60 seconds. With the help of statistical methods 2 groups of fleas were distinguished - rapid and slow feeding. This phenomenon was observed both in X. gerbilli minax and in C. laeviceps. It is interesting that such a phenomenon was observed by other authors during the feeding of blood-sucking mosquitoes on an animal (O'Rourke, 1956) and during the feeding of A. cheopis, X. gerbilli minax and Ctenophthalmus dolichus on animals (Balashov et al., 1961), which speaks for the similarity of processes of natural and forced feeding. The disclosure of rapid and slow feeding specimens of fleas is connected with the different mechanism of drawing in of blood: some drink directly from the capillary and others drink blood which has emerged from the vessels into the surrounding tissue. Such proposals were expressed earlier (Patton, Ivans, 1929; Snodgrass, 1944). At present they have received factual confirmation.

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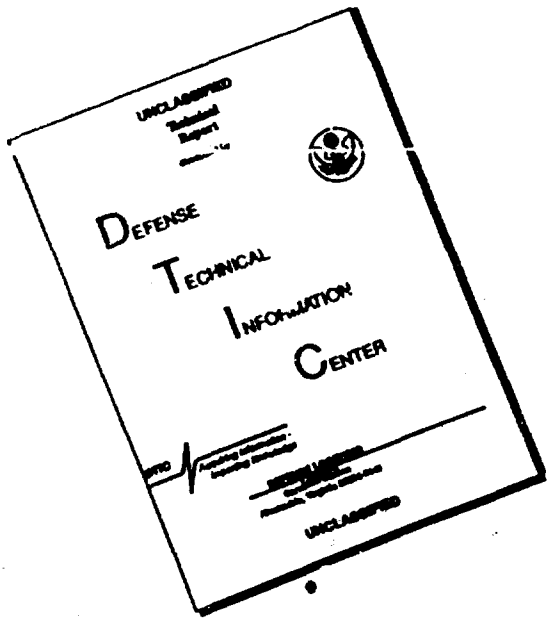

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On both groups of fleas we made a comparison of the rate of feeding depending on the nature of the nutrient liquid. It is clear from the data in Table 1 that the females of both species of fleas feed noticeably more rapidly than the males, which is apparently connected with the more powerful sucking apparatus. It turned out that fleas of both species could be fed with greater or lesser success with practically any infecting liquid, including pure Nottinger broth. It seems to us that this fact is particularly important, since it makes it possible to consider that in the future there is the possibility of the proportioned infection of fleas with liquid which contains not only broth and microbes, but also other ingredients which are capable of promoting or oppressing the activity of microbes in fleas. Of the 3 species studied the easiest to feed was X. merbilli minax.

As can be seen from the data in Table 1 the rate of satiation depends, though not always expressed sharply, on the nature of the liquid. Thus the fleas fed slowly when they were given broth, but they fed noticeably more rapidly on liquids containing blood.

Of the latter, liquid was engorged relatively more rapidly when it contained the blood of rodents in comparison with the blood of a horse, and among the rodents it was most rapidly with the blood of guinea pigs, then white rats and gerbils. This also had an influence on the volumes of engorged liquid.

There were no particular differences in the rate of consumption of hemolyzed and defibrinated blood, though, based on visual observations, fleas begin to drink defibrinated blood more willingly, nevertheless they drink it more slowly, than hemolyzed, which is apparently connected with the formation of "packets" of erythrocytes in the capillary and with their rapid settling in the micropipet. Judging on the rate of feeding it is preferable to use hemolyzed blood, since it is of greater practical convenience for rated feeding. The absolute values of feeding rates (from several seconds to several minutes) agree quite accurately with data from other authors (Balashov et al., 1961).

In passing we made observations on the rate of feeding by X. m. minax fleas on unheated liquid at room temperature (21°) and liquid heated up to 37° in a microincubator, into which the end of the micropipet was inserted. The heated liquid was taken in approximately 2.8 times more rapidly than unheated liquid.

Absorption of liquid by fleas takes place very nonuniformly. As can be seen from Fig. 1, in the first seconds of feeding the fleas drink more than a third of all the liquid. This process is particularly swift for C. laeviceps fleas, which, as was seen from Table 1, feed considerably more rapidly than X. m. minax. Then a slowing down of feeding is observed, and sometimes even a pause lasting up to several seconds, after which feeding is resumed.

Table 1

Rate of satiation of fleas (in seconds) during forced feeding of nutrient liquid with the addition of blood of various animals

(a) Животное — донор	*Xenopsylla gerbilli minax*				*Ceratophyllus laeviceps*			
	(b) характер добавленной крови							
	гемолизированная (c)		дефибринированная (d)		гемолизированная (c)		дефибринированная (d)	
	самцы (e)	самки (f)	самцы (e)	самки (f)	самцы (e)	самки (f)	самцы (e)	самки (f)
(g) Лошадь.	— (B) 139.0±20.3 (M)	30.5±9.4 149.1±31.6	60.0 135.0±21.4	34.3±9.3 80.0	• —	• —	30.0 —	19.1±6.1 —
(h) Песчанка полуденная.	55.0±3.2 (B) 120±25.4 (M)	30.8±2.5 85.0±5.0	35.0 101.5±7.5	44.0±6.7 —	51.0±11.3 154±40.6	31.5±9.1 —	— —	38.6±11.8 —
(i) Белая крыса.	— (B) 117.5±21.9 (M)	35.0±6.5 —	— —	— —	— —	21.2±2.5 —	— —	24.0±7.6 —
(j) Морская свинка.	— (B) 171±19.8 (M)	24.0±7.0 76.2±0.6	45.0±0.1 —	32.0±9.4 113.0	30.0±5.9 —	16.0 75.0	26.0 —	23.0±1.41 —
(k) Контроль — бульон Хоттингера.	(e) Самцы 105±43.5 (f) Самки 55.0; 90.0				(l) Самцы не питались (f) Самки 15.5±0.2; 105—540 **			

Note. In parentheses: *B* - rapidly feeding fleas, engorging 0.045 ml of liquid in less than one minute; *M* - slow feeding fleas, feeding for more than one minute.

* Denial of feeding (in the tests of 25 specimens, males and females).

** Figures should be reduced by approximately 2.8 times, since based on technical reasons the feeding was conducted with unheated liquid.

Key: (a) animal donor; (b) nature of blood added; (c) hemolyzed; (d) defibrinated; (e) males; (f) females; (g) horse; (h) midday gerbil; (i) white rat; (j) Guinea pig; (k) Control - Hottinger broth; (l) males did not feed.

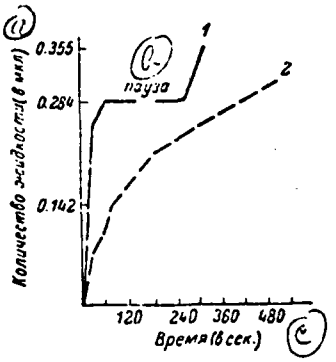

Fig. 1. Dynamics of satiation of female fleas which were infected with liquid containing defibrinated blood.
1 - C. laeviceps, 2 - X. gerbilli minax.
Key: (a) Amount of liquid (in microliters); (b) pause; (c) Time (in seconds).

During the time of the pause we were able to observe a jerky shift in the column of liquid in the capillary backwards - forward, creating the impression that the flea both sucks in and expels a small amount of liquid. It is possible that this is a phenomenon, analogous to that observed by Yu. S. Balashov and associates (1961), of natural regurgitation of fleas.

Uninterrupted observation in a binocular of the feeding of individual specimens of various species of fleas showed that in the majority of cases C. laeviceps fed better and more rapidly when they were as if suspended by the proboscis, the piercing-sucking portion of which was inserted in the capillary (Fig. 2a). It is interesting that we were able to observe the turning of the epipharynx around its own axis within the capillary, just as this was observed in animal tissues by Deoras and Joshee (1961), and also to see that the process of feeding can continue in the event of the very strong bending of the epipharynx at any angle to the side or upwards (forward), which also agrees with the data of these authors. The position of the laciniae does not have any significant importance. Feeding, as this was shown by Lavoipierre and Hamachi (1961), actually takes place through the epipharynx. The fleas sucked in liquid even in those cases when the laciniae were not even in the capillary. We were also able to observe that the feeding of fleas, X. g. minax in particular (Fig. 2, b), is possible without immersion of the proboscis in the capillary; in some cases it was sufficient to have contact with the tip of the epipharynx against the meniscus of the liquid in the capillary in order for feeding to begin. This observation is very important to us, since it indicates the possibility of "sublysing" of liquid from drippings, including from drippings from infected blood or the excrements of another strongly engorged flea. This assumption

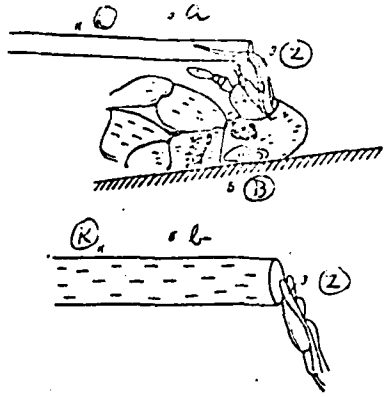

Fig. 2. a - position of proboscis of C. laeviceps during feeding through a capillary, b - position of proboscis of X. gerbilli minax during lysing of liquid; K - capillary, Z - epipharynx, B - vacuum holder.

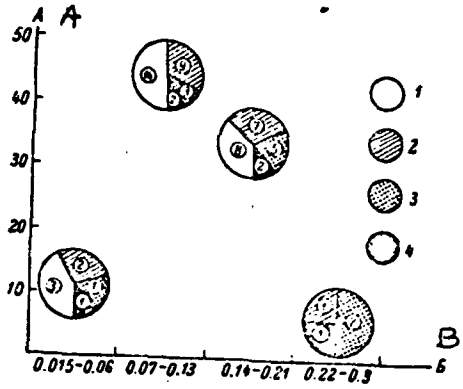

Fig. 3. Survival of X. g. minax females depending on the amount of absorbed liquid (amount of microbes). A - number of fleas (in % to total number of specimens) which drank various doses of liquid; B - amount of absorbed nutrient liquid in microliters. Number of specimens which survived for: 1 - 1-8, 2 - 9-16, 3 - 17-24, and 4 - 25-32 days.

of ours was indirectly confirmed by the following observations: in some of the X. g. minax fleas (in approximately 6-7 cases out of 100) which were taken from the insectarium for experiments on the forced feeding, in the IV—V stage of digestion of blood the tip of the proboscis turned out to be contaminated with a dried droplet of blood.

Sometimes part of the drop was on the coxae of the forward extremities. When the proboscis was inserted into a capillary with Mottinger broth the drop was dissolved, coloring it. Such a phenomenon may be explained only by the "sublysing" by starving fleas of the drippings of excrements of other fleas. And the excrements of contaminated fleas, as is known (Golov and Ioff, 1928), frequently, though irregularly, contain viable plague microbes.

For appraising the possible degree of contamination of this or that liquid it is important to know not only how the fleas feed, but also how much liquid they absorb (Fig. 3).

Forced feeding of A. g. minax and C. laeviceps is possible, as can be seen, with all the liquids used in the tests with the exception of a mixture containing the hemolyzed blood of a horse, which C. laeviceps fleas refused to drink. As can be seen from Fig. 3 it is possible to force feed A. g. minax fleas in a greater percentage of cases than C. laeviceps, which rejected sucking more often.

It is necessary to stipulate that when the experimenter has sufficient time and patience it is possible to gain a considerable increase in the percentage of feeding specimens: in our tests we considered as "rejecting feeding" those fleas which did not start to drink in the course of 5--7 minutes. Meanwhile some specimens, C. laeviceps especially often, begin to drink (sometimes very intensively) in the 7th, 10th, and even 15th minute. The method of rated feeding permits the direct measurement of the weight of the liquid sucked in by one specimen (volume x specific weight = weight of absorption). All previous methods were gravimetric and one could weigh only batches of fleas and find only the average weight.

Females as a rule take in greater volumes of blood than males. A similar phenomenon was observed earlier both for fleas (Balashov et al., 1965; Kunitskiy, 1961; Bryukhanova et al., 1961) and for other blood-sucking insects in which both sexes are blood-sucking, for example, in clones of the genera Cimex and Triatoma (House, 1958).

Actually fleas can feed on practically any liquid, even water, as was noted by Kartman (1954), or physiological solution (our observations). However, as the experiments showed, both species of fleas being tested turned out to be far from indifferent to "taste" of the liquid which was given to them. This was expressed both in a decrease of the relative amount of rejections and in an increase in the volume of liquid taken in by the fleas. Thus in practically all cases the fleas more readily drank liquid with the addition of blood, while with the blood of rodents it was more willingly than with the blood of horses. It is more difficult to trace a clear line in the "taste" preferences to the blood of various rodents. However, the impression is created that out of the number of tested specimens the fleas fed most willingly on liquid with the addition

of guinea pig blood in it, both hemolyzed and defibrinated. These findings as if supplement our observations concerning the rapid rate of engorgement by fleas of a liquid with the addition of blood primarily of this rodent. The results of our observations have more than just a purely theoretical significance, since it is known that plague microbes survive best and are preserved longer in the organism of fleas when the latter have fed on the contaminated blood of mainly the pig in comparison, for example, with the blood of white mice (1.8 times) - (Tiflov, 1964). It is all the more important that the blood of guinea pigs is obtained easily in the laboratory.

For the proposed method of forced feeding through a capillary there is also practical significance in the circumstance that there is no expressed difference in feeding with liquid with added hemolyzed or defribinated blood. It is true that during feeding with defibrinated blood specimens predominate which absorb considerable volumes of liquid, however, to a considerable degree this advantage brings to nothing the difficulties (which we already mentioned) in working with liquid containing non-destroyed erythrocytes.

For judging the preferableness of this or that blood it is also necessary to consider the maximum volume of liquid which is engorged by individual specimens. Thus the largest amount of liquid (0.3—0.5 microliters) was drank by individual specimens of C. laeviceps females which had fed on liquid containing the hemolyzed blood of a gerbil and the defibrinated blood of a guinea pig, and X. g. minax - the defibrinated blood of a white rat and guinea pig. However, the use of this criterion for appraising the preferableness of this or that mixture is still limited by a scarcity of observations.

Data concerning the feeding of X. cheopis were not included by us due to the very small number of observations: out of 5 females one fed on liquid with defibrinated rat blood, and out of 3 females - one with hemolyzed. The first one drank all told 0.22 microliters at a rate of engorgement of 0.045 microliters in 55 seconds, and the second - 0.17 microliters at a rate of 16 seconds. The first one lived for 19 days and the second - 6 days. By the moment of death both were free of microbes.

We followed contaminated fleas and obtained preliminary data concerning the fate of the plague microbe in the organism of contaminated specimens. In the tests we used both starving specimens of known age and fleas in the IV--Vth stage of blood digestion (Ioff, 1949), the exact age of which was unknown. After the forced feeding of fleas they were fed on white mice once every 3—4 days and kept at 21—24° and 85—90% relative humidity. Fleas which died were investigated for the presence of plague microbes in them.

As can be seen from Table 2 the number of fleas which perished on the 5th--12th day, i.e., lived (on the average) for a week, comprised 87% for X. g. minax fleas which had not fed once. For fleas

of this species which were taken in the IV—Vth stage of blood digestion it was 70.5%, and finally for C. laeviceps it was 88.6%. The number of fleas which died in the first 4 days was calculated by individuals. The males, as it should have been expected, survived more poorly in all cases.

Table 2

Dying off of fleas which had preliminarily been forced fed with a culture of plague

(a) Наблюдения в (сутках)	Количество блох, погибших в разные сроки (b)					
	Xenopsylla gerbilli minax				Ceratophyllus laeviceps	
	ни разу не питавшиеся блохи (c)		блохи, взятые в опыты на IV–V стадии переваривания (d)			
	♂	♀	♂	♀	♂	♀
1–4	—	—	4	2	3	3
5–8	2	3	12	30	15	15
9–12	2	6	8	12	—	5
13–16	1	—	8	4	—	1
17–20	—	—	2	—	—	—
21–24	—	—	1	—	—	—
25–28	—	—	—	3	—	—
29–32	—	1	—	2	—	—
(e) Всего под наблюдением	5	10	35	53	18	24
(f) Из них оказались заражёнными к моменту гибели	2	1 (10%)	1 (2.8%)	6 (11.3%)	—	—

Key: (a) Observations (in days); (b) Number of fleas, died in various periods; (c) fleas which did not feed once; (d) fleas taken in tests in the IV—Vth stage of digestion; (e) Total under observations; (f) Of these, the number which turned out to be infected at the time of death.

Under laboratory conditions 50% of uninfected C. laeviceps died off under similar conditions of feeding and upkeep also in a week on the average (Kunitskiy, 1966). X. g. minax lived somewhat longer, up to a month, at 25—27° and 80% humidity (Bogytova, 1966). The presence of plague microbe in the fleas somewhat reduced the period of life of the flea, therefore our findings show that the death of fleas is not found in any noticeable dependence on the procedure of forced feeding, since usually fleas of these species live for similar periods at a temperature of 21—24°. By the time of death the majority of fleas turned out to be free from microbes, and only 10—11% of X. g. minax females turned out to be infected. We will attempt to reveal the reasons for this phenomenon below.

In an analysis of the survival rate there was interest in the fact of the detection of a dependence between the amount of initially

engorged contaminating liquid and the life duration of the infected specimens.

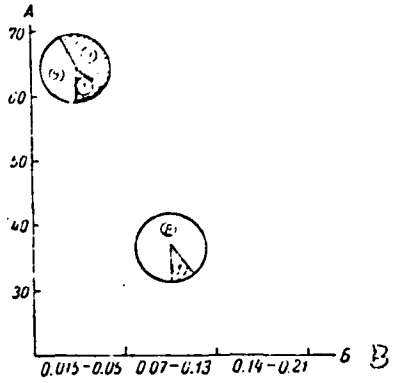

Figure 4. Survival of male X. g. minax depending on the amount of engorged liquid (number of microbes).
Legend same as in Fig. 3.

It follows from Figure 4, which presents the findings for females [this does not agree with the figure caption], that the number of long living specimens does not drop with an increase in the amount of engorged nutrient liquid (and, consequently, the number of microbes, since in all cases for the feeding of fleas feeding mixtures were used which contained 1 billion microbial bodies in 1 ml). Such a nature of survival of fleas is possibly connected with the rapid riddance of plague microbe by females. In passing we will note that there was a predominance of females which had taken in average amounts of liquid: from 0.07 to 0.21 microliters. A somewhat different picture was shown by the feeding and survival of X. g. minax males (Fig. 4). First of all, there was a predominance of specimens which had taken in large amounts of liquid, and, secondly, among specimens which had absorbed large doses of microbes the survival rate was lower than among those which were less intensively infected. To judge the causes of these phenomena is difficult due to the insufficient amount of observations, though it is not excluded that mechanisms of purification from microbes are less perfected in males than in females.

For a judgment of the fate of microbes in the organism of fleas and of the ratio of the calculated number of microbes supplied in the nutrient liquid to the amount actually absorbed, observations were carried out in which X. g. minax fleas, after various intervals of time, were investigated by means of titration and seeding on agar. These data are summarized in Table 3.

The existing assumption of Duncan (1926) and Tiflov (1964) that in the organism of fleas, similar to other Arthropoda, there exists a certain nonspecific bactericide which promotes the self-sterilization of their organism in response to the introduction of microbes is confirmed by our data.

Table 3

Detection of P. pestis microbes in the organism of forced fed X. cheopis flees

а штамм	б Пол блох	в Количество зараженных блох	г Количество блох, у которых обнаружены микробы	д Среднее количество микробных тел в одной зараженной блохе	е Количество микробных тел, обнаруженных посевом (в)	ж Через 1,5 часа при 21—24°	з Через 3—5 час. при 7—8°
EV	♂ самцы	3	1	10000—50000	50	—	—
		1	1	50000	1000—1500	—	—
	♀ самки	1	1	100000	60	—	—
		1	1	250000	250—500	—	
		6	0	500000—2500000	—	0	
		1	1	37000	—	—	20000
		1	1	80000	—	—	30000
		1	1	180000	—	—	85000
161	♀ самки	1	1	15000	10000	—	—
		5	1	50000	—	200	—

Note: Seeding of microbial bodies not in 3, but in 1.5 hours.
Key: (a) Strain; (b) Sex of fleas; (c) Number of infected fleas; (d) Number of fleas in which microbes were detected; (e) Calculated amount of microbial bodies absorbed by a specimen; (f) Amount of microbial bodies detected by seeding in; (g) 1.5 hours at 21—24°; (h) 3—5 hours at 7—8°; (i) Males; (j) Females; (k) 15—30 min.

Actually, even in the seedings from fleas which had received tens and even hundreds of microbial bodies with the nutrient liquid, in 15—30 minutes there is a reduction in their number by from 100 to 1000 times, and in several hours in the overwhelming number of cases the fleas infected with the EV strain turned out to be sterile. We see an analogous picture in the work of Hartman and associates (1956), in which a sharp reduction is shown in the number of microbial bodies in X. cheopis fleas in the course of the first day after the contaminated feeding. It is interesting that in complete conformity with the data of Tiflov the microbes of virulent strain No 161 were preserved better than the EV vaccine strain in fleas. In accordance with the data of Duncan the hypothetic bactericidae did not have an effect at a reduced temperature, and in our tests at 7—8° practically the same amount of microbes was preserved in fleas as was introduced. In all cases figures were obtained which were on the same order as calculated, and in one - a practically ideal conformity: 80 and 80 thousand microbial bodies. It is necessary to take into account that the tests bore a preliminary nature and inoculation with a dilution of 1/10,000 was made on one Petri dish with agar, therefore it was especially important to obtain a conformity on orders of numbers reflecting the number of microbial bodies, which was achieved in those experiments.

The work carried out, in our opinion, is the basis for the subsequent study of the fate of microbes in the organism of carriers - fleas, the reactions of fleas to the introduction of various numbers of microbial bodies, and the adaptational mechanisms of the microbes themselves.

Conclusions

1. Studies were made of the rate of feeding and degree of engorgement of X. cerbilli minax and C. laeviceps fleas during forced feeding through capillaries. Differences were shown in the rate of absorption and volume of infected liquid taken in depending on its qualitative composition.

2. Observations were made of the survival rate of fleas which had been forced fed with plague microbes; it was established that the death of fleas as a result of the procedure of feeding did not increase noticeably in comparison with normal.

3. The relationship of calculated number of microbial bodies absorbed by fleas to those actually contained in them was established; a rapid reduction was revealed in the number of microbes in the organism of fleas at 21—24° in the first hours after they enter the organism of the flea.

Bibliography

Alekseyev, A. N., 1965. Forced rated feeding of insects. Med. parazitol. i parazitarn. bol., 4:467--471.

Alekseyev, A. N., Bibikova, V. A., and Khrustselevskaya, L. M., 1967. Method for the individual forced rated infection of fleas with plague microbes. Parazitol., 1(2):176--179.

Balashov, Yu. S., Bibikova, V. A., Murzakhmetova, K., and Polunina, O. A., 1961. Fleas as a medium of residence for plague microbes. Report 1. Feeding and digestion of blood in non-contaminated fleas. Materials from the extended scientific conference celebrating the 40th anniversary of Kazakhstan. Alma-Ata : 27--30.

Balashov, Yu. S., Bibikova, V. A., Murzakhmetova, K., and Polunina, O. A., 1965. Feeding and disruption of valvular function of the gizzard of fleas. Med. parazitol. i parazitarn. bol., 4:471--476.

Zgytova, S. I., 1966. Period of life of great gerbil fleas Xenopsylla cerbilli minax, Wagn. 1910 in an experiment in connection with conditions of the medium and feeding. Transaction of the 6th Conference on Naturally Focal Diseases and Problems of Parasitology, Central Asia and Kazakhstan.

Bibikova, V. A., Osadchaya, L. M., and Khrustselevskaya, K. M., 1961. The study of mutability of the plague microbe in fleas. Materials from the extended scientific conference celebrating the 40th anniversary of Kazakhstan : 30--31.

Bibikova, V. A. and Khrustselevskaya, N. M., 1965. One of the trends in the study of fleas and the plague causative agent. Materials of the 4th scientific conference on the natural focalness and prophylaxis of plague. Alma-Ata : 83--84.

Bryukhanova, L. V., Bardar, Ye. A., and Levi, M. I., 1961. Method for determination of the amount of blood engorged by fleas. Tr. nauchno-issl. protivochumn. Inst. Kavkaza i Zakavkaz'ya. 5, 23--33.

Golov, D. A. and Ioff, I. G., 1928. Influence of various conditions on the preservation of the plague microbe in the organism of fleas and various stages of their development. Tr. 1-go Vsesoyuzn. protivochumn. soveshch., Saratov ; 158--180.

Ioff, I. G., 1949. Aphaniptera of Kirghizia, 1--242.

Kunitskiy, V. N., 1961. The question of feeding of gerbil fleas Ceratophyllus laeviceps Wagn., 1908. Materials from the extended scientific conference celebrating the 40th anniversary of Kazakhstan, Alma-Ata : 107--109.

Kunitskiy, V. N., 1966. Ecological-geographical outline of fleas of south-western Azerbaydzhan in connection with their significance in the natural focalness of plague. Thesis for candidate of biological sciences, Alma-Ata.

Tiflov, V. Ye., 1964. Fate of bacterial cultures in the organism of fleas. In the collection: Ectoparasites, 4:181--198.

Dooras, P. Y. and Joshee A. K., 1961. A note on the feeding mechanism of X. cheopis. Current Sce., 30(12); 465--466.

Duncan, I., 1926. On a bactericidal principle present in the alimentary canal of insects and arachnids. Parasitology, 18(1) : 238--252.

House, H., 1958. Nutritional requirements of insects associated with animal parasitism. Expl. Parasitol., 7 : 555--609.

Hartman, L., 1954. Studies on Pasteurella pestis in Fleas. I. An apparatus for the experimental feeding of Fleas. Expl. Parasitol., 3(6) : 525--537.

Hartman, L., Prince, F. M., and Quan, S. F., 1956. III. Comparative Plague-Vector efficiency of Xenopsylla vexabilis howaiiensis and Xenopsylla cheopis. Bull.World Health Org., 14(4) : 684--704.

Lavoipierre, M., and Hamachi, M., 1961. An apparatus for observations on feeding mechanism of the Flea. Nature, 192, (4806) : 998--999.

O'Rourke, F. J., 1956. Observations on pool and capillary feeding in Aedes aegypti (L). Nature, 177, (4519) : 1087--1088.

Patton, W. S. and Evans, A. M., 1929. Insects, Ticks, Mites, and Venomous Animals. Part 1 : 1--735.

Snodgrass, R. E., 1944. The feeding apparatus of biting and sucking insects affecting man and animals. Smith. Inst. Publ. Misc Collections, 104(7) : 1--113.